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Enhanced lipase production of *Fusarium verticillioides* by using response surface methodology and wastewater pretreatment application

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Abstract

Lipases are enzymes able to hydrolyze the ester bond of long-chain acylglycerols. This work aimed to investigate the production, optimization and characterization of *Fusarium verticillioides* lipase and its application on fatty acid production and fat particles hydrolysis. Response surface methodology (RSM) was employed to optimize the production. Adams medium was used in the submerged fermentation, at 30°C for 96h. Sunflower oil and peptone were the best carbon and nitrogen sources. The optimized medium showed an activity 2-fold higher than the original medium and was composed by 0.15 % KH_2PO_4, 0.025 % $MgSO_4$, 0.3 % peptone and 1 % sunflower oil. The optimal temperature and pH was 45°C and 5.5, respectively. The enzyme had a remarkable stability in a pH range of 4.0-8.0. The hydrolysis of vegetable oils showed sunflower oil as better hydrolyzed compared to others. A fat reduction of 3-fold was evaluated in the slaughterhouse pretreatment.

Keywords: *Fusarium verticillioides*, lipases, surface response, wastewater pretreatment.

Introduction

Lipases (EC 3.1.1.3; triacylglycerol acylhydrolases) are hydrolases with a great ability to perform very specific chemical transformations. Due to their availability and stability, lipases hydrolyze triacylglycerol with long fatty acid chains liberating free fatty acids and glycerols and it has made this group indispensable to biotechnology industry. Consequently, they have emerged as one of the leading biocatalyst capable to realize synthesis reactions, transesterification, esterification and, the most common, hydrolysis of triacylglycerol, contributing to a capital underexploited of bioindustry (Singh & Mukhopadhyay, 2012).

Owing to this incredible capacity of reactions, the increasing of lipase production during fermentation processes, aiming high activity with low cost and low residues is industrially attractive. However, few efforts have been made to improve this production by varying the medium components and by verifying internal interactions during the cultivation process. Thereby, the optimization of medium culture using response surface methodology (RSM) is an increasing field in biotechnology and an alternative secure in order to improve the fermentation process, in which many studies have been done (Andrade et al., 2013; Kaushik et al., 2010; Kumari et al., 2009; Liu & Zhang, 2011; Rajendran & Thangavelu, 2009; Ramani et al., 2013).

Among the microorganisms, fungi are considered one of the best lipase producers. They generally produce this enzyme by submerged fermentation processes (SmF) using a culture medium compound by yeast extract, peptone, soy meal, beef extract or corn steep liquor, with oil or waste residues as lipase inducers (Kantak et al., 2011; Mayordomo et al., 2000; Ramani et al., 2013).

The optimization using statistical analysis and RSM are important tools to determine the optimal medium culture conditions and a strategy for solving problems in industrial fermentation processes (Chandrashekar et al., 1999); for the reason that when the medium components are analyzed separately, the concentration of each compound became time-consuming (Veerapagu et al., 2013). In this context, the use of mathematical models facilitates data analysis and provides an increase in the productivity and yield of many enzymes production (Chandrashekar et al., 1999; Rajendran & Thangavelu, 2009).

The high productivity and the enzyme low cost is indispensable to the most of environmental applications, however the use of pure and commercial enzymes are expensive. Thus, the use of crude extracts with high lipase activity is required. Pretreatment procedures to hydrolyze and

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dissolve fats are known as an important step to improve the biological degradation of fatty wastewater, because it can accelerate the process by decreasing the fat adsorption to the surface of the anaerobic sludge and not limiting the transport of the soluble substrate to the biomass (Perle et al., 1995; Valladao et al., 2009).

Effluents from slaughterhouses have been a potential application of enzyme treatments, because it contains high concentrations of biodegradable organic matter, most of which consists of lipids and proteins with low degradability. Consequently, there is a necessity to reduce fat, oil and protein from these and from others oily wastewaters, once the reduction the organic matter contribute to a cleaner effluent (Valladao et al., 2011).

Therefore, the goals of this study were increase the lipase productivity in SmF and apply the enzymatic crude extract in environmental treatments. The effect of various medium components on lipase production from *F. verticillioides* was analyzed using central composite rotational design (CCRD) statistical experimental design. The crude extract was characterized biochemically and its effects were analyzed in slaughterhouse pretreatment and oil hydrolysis.

Materials and Methods

Microorganism and culture maintenance

The fungal strain was isolated from Buriti seeds and was identified as *Fusarium verticillioides* by Universidade Federal de Pernambuco (UFP), Department of Mycology, in Pernambuco, Brazil. F. verticillioides stock culture was maintained on oatmeal agar medium, at 4°C.

Submerged fermentation conditions

The submerged cultures were incubated in 150 ml Erlenmeyer flasks containing 25 ml of liquid medium on a rotary shaker (100 rpm), at 30°C. The lipase production and mycelium growth were evaluated at 25, 30, 35 and 40°C. Initially, different mineral salt medium were tested for 120 h, 100 rpm: Adams (Adams, 1990), Khanna (Khanna et al., 1995), SR (Rizzatti et al., 2001), Czapek (Wiseman, 1975), Vogel (Vogel, 1964) and M5 (Peralta et al., 1990). After chosen the liquid medium, the time cultivation was varied from 48 to 240 hours.

In order to select the best carbon source, 1% of each oil was tested: soybean oil, canola oil, sunflower oil, macauba pulp oil, macaúba seed oil, macaúba almond oil, olive oil, neem oil, palm oil, sesame oil, residue of diesel industry, pequi oil, castor oil, corn oil, baru seed powder (Dipteryx alata) and crushed jatropha seeds. Effect of nitrogen sources (0.2%) on the lipase production was also studied by replacing the original nitrogen sources by urea, wheat bran, ammonium acetate, peptone (control), monobasic potassium phosphate, rye bran, bibasic potassium phosphate, ammonium nitrate, yeast extract and ammonium chloride.

Experimental design for lipase production using RSM

In order to determine the optimal fermentation conditions to increase the enzyme production, a 2^4 central composite rotational design (CCRD) was employed to study the interaction of four variables: KH_2PO_4 (X_1), $MgSO_4$ (X_2), peptone (X_3) and sunflower oil (X_4). Four central points were included in this study. The coded and real values of the variables are shown in Table 1. A regression analysis was performed on the data obtained from the design experiments. Analysis of variance and multiple regression analysis were performed at $p<0.05$, using STATISTICA Version 8.0 (Stat Soft Inc., OK, USA). The lipase activity was taken as dependent variable or response. A polynomial equation (Eq. 1) was assumed to correlate the relationship between the variables and the response:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{44} {X_4}^2 + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{14} X_{14} + \beta_{23} X_{23} + \beta_{24} X_{24} + \beta_{34} X_{34} \quad (1)$$

Where, Y is the predicted response; β_0 the intercept; β_1, β_2, β_3, β_4 the linear coefficient; β_{11}, β_{22}, β_{33}, β_{44} the squared coefficients; and β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34}, the interaction coefficients.

Table 1: Experimental range and levels of the five independent variables

	Coded variables				
Variables (%)	-α	-1	0	+1	+α
KH_2PO_4	0	0,05	0,1	0,15	0,2
$MgSO_4$	0	0,025	0,05	0,075	0,1
Peptone	0	0,1	0,2	0,3	0,4
Sunflower oil	0,25	0,5	0,75	1	1,25

Lipase activity and protein quantification

Lipase activity in the culture filtrate was determined using *p*-nitrophenyl palmitate (pNPP) as the substrate (Pencreach & Baratti, 1996). The reaction was carried out initially in McIlvaine buffer (pH 5.5), at 40 °C (McIlvaine, 1921). After the enzyme characterization the activity was evaluated according to the best enzyme conditions. One unit (U) of lipase activity was defined as the amount of enzyme required to release 1 µM of *p*-nitrophenol per ml per minute.

The titration method determined the free fatty acids concentrations (Polizelli et al., 2008). The reaction mixture contained 34.3 % vegetable oil, 45.7 % 50 mM sodium phosphate buffer (pH 5.5), 2.9 % Triton X-100 and 17.1 % crude extract. The reaction was stopped by adding acetone: ethanol (1:1) to denature the enzyme and effectively stopping the reaction. The final analysis was performed in a potentiometric titration using 50 mM KOH (*end point* pH 8.0). A standard curve with oleic acid was determined and the fatty acids released during hydrolysis were obtained in mM of fatty acids. Protein concentrations were measured using the dye binding method, with BSA as standard (Bradford, 1976).

Crude extract characterization

Determination of optimal temperature and pH

In order to determine the optimal conditions to the lipase activity, the influence of temperature varying from 25 to 70 °C and the pH range from 3.0 to 10.0, was evaluated using pNPP as substrate. The residual lipase activity was assayed under standard conditions.

Determination of Thermal, pH and solvent Stabilities

The lipase was incubated at the following temperatures: 30, 40, 50 and 60°C and the pH range were varied from 3 to 10, for 180 minutes and 24 h, respectively. The stability in organic solvents was evaluated by incubating the crude extract in

hexane, methanol, ethanol, isopropanol, DMSO, butanol and acetonitrile (50 %, v/v), at 25 °C for 24 h. The residual lipase activity was assayed under standard conditions.

Vegetable oil hydrolysis

The hydrolysis was performed with diverse oils: canola, olive, sesame, cotton, castor, pequi, macauba pulp, macauba seed pulp, sunflower, corn, soybean and soybean fried (used to fry potatoes). The crude extract contained approximately 12 U/mL. The reaction was incubated at 45 °C, 200 rpm, for 18h. The fatty acids released were determined by titrimetric method. A blank titration was done as control sample for each oil sample tested. The hydrolysis conversion was calculated as described by Abdelmoz et al. (2013).

Oil soapstock hydrolysis from soybean oil refining

The reaction was performed by mixing 6 g oil soapstock, 16 mL 0.1 M sodium phosphate buffer (pH 5.5), 0.5 mL Triton X-100 and 3 mL crude extract. The mixture was incubated at 45°C, 200 rpm, for 18 h. The lipase activity was determined by titration through the KOH volume. A blank titration was done as control sample. The hydrolysis conversion was calculated as described by Abdelmoez et al. (2013). The fatty acid average of soybean oil was used to this conversion.

Fat particles degradation on slaughterhouse wastewater pretreatment

Wastewater was collected from a slaughterhouse processing industry (Santa Barbara D'Oeste SP, Brazil). It was filtered to eliminate bigger particles and autoclaved to avoid any interferences by the wastewater microflora. The filtered wastewater contained only fat solids in dissolved form (> 0.1 mm). The hydrolysis experiments were conducted as described by Masse et al. (2001). Fat (0.5 ± 0.02 g) and tallow (0.7 ± 0.05 g) particles were cut into small peace and immersed in McIlvaine buffer (pH 5.5). The hydrolysis consisted of 22% filtered wastewater, 22% buffer containing fat or tallow particles immersed, 44% crude extract (lipase activity 12 U/mL) and 12% Triton X-100. Adding particles to the slaughterhouse wastewater could control the size and composition of the mixture (Masse et al., 2001b). The reaction was carried out at 45 °C, 100 rpm, for 72 h. After hydrolysis the particles were weighted and titrated against KOH (Masse et al., 2001b).

Results and Discussion

F. verticillioiodes lipase production

Fungal lipases are generally produced in submerged fermentation due to its facility to be purified and generate lower residues than solid state fermentation. The selection of the liquid medium in submerged fermentation, growth and production temperature and an appropriate incubation time are important parameters to enhance the lipase production. Maximum enzyme production was obtained with Adams medium, after 96 h of incubation at 30 °C, 100 rpm (Figure 1 A and B). *F. verticillioides* could be considered a mesophilic fungal as it reached its best fungal growth and lipase production at 30°C (Figure 1 C and D). Kantak et al. (2011) reported that *Rhizopus sp* reached the best lipase production at 30°C, for 72 h of growth in JK1in a basal medium. Silva et al. (2005) obtained 50 h as the best fermentation time culture for lipase production by *Aspergillus nidulans* e *Metarhizium anisopliae*, respectively.

The addition of fat-related carbon sources to the culture medium induces the lipase production. In general, oils play an important role to induce this production. They have been used as lipase inducer in SmF by many authors (Andrade et al., 2013; Basheer et al., 2011; Colin et al., 2010; Lima et al., 2003; Maia et al., 2001; Messias et al., 2009; Silva et al., 2005; Singh & Mukhopadhyay, 2012). The *F. verticillioides* lipase activity was highly enhanced mainly by sunflower oil; however soy and canola oil also had a good production (Figure 2A). Silva et al. (2005) had similar results with sunflower, olive and soybean oil. Neem oil and baru powder presented the worst results, possibly due to the fungicide characteristic of neem oil and probably because the baru powder composition has a lower oil concentration available as inducer. Peptone was obtained as the best nitrogen source, being 2-fold higher than the second best source, rice bran. However, bibasic potassium phosphate, ammonium chloride and urea decreased or inhibited lipase production (Figure 2B). *Penicillium aurantiogriseum* produced higher lipase activity with olive oil in the presence of ammonium sulfate as nitrogen source (Lima et al., 2003).

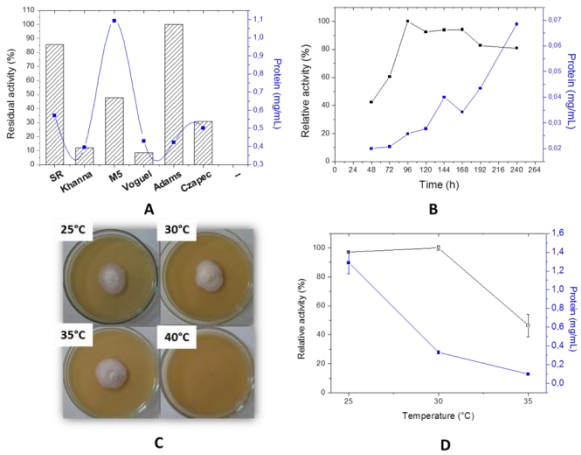

Figure1: Influence of nutrient medium (A) and incubation time (B) and temperature on (C) the growth and (D) lipase production. Symbols: (A) gray bars represent relative activity and black squares protein (B) Black squares represent the relative activity and blue squares represent the protein; (D) filled and blue squares - protein (mg/mL); open and black squares - lipase activity.

Compounds as surfactants can increase cell permeability, facilitating the export of several molecules across the cell membrane and the contact between enzyme and substrate (Silva et al., 2005). Hence, it was investigated the influence of some surfactants on lipase production (PEG 4000 and 8000, SDS, Tween 20 and 80, SDS and Triton X-100), but no one could increase the enzyme activity (data not shown). Aiming the same objective, different glucose concentrations (0.05 to 0.2 %) were studied and higher glucose concentrations (0.02 %) decreased the lipase activity (data not shown).

Optimization of the fermentative medium components

Optimization of the culture conditions is an effective and timesaving tool to evaluate the significance of some fermentative parameters in an experiment, and it could increase the production many folds as compared to the un-optimized conditions. Table 2 shows the central composite design matrix presenting the coded values and the response (lipase production).

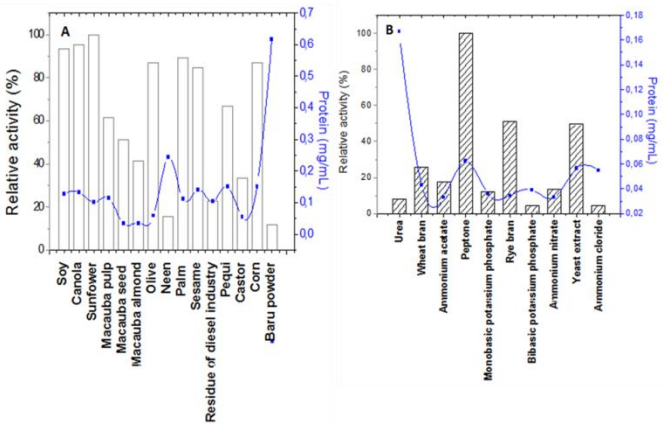

Figure 2 Effect of (A) different oils as carbon sources and (B) nitrogen sources on lipase production. Symbols: Gray bars represent the residual activities and blue squares, protein.

Table 2: Central composite design matrix (including the central points) for lipase production as response.

Assays	Parameters				Lipase activity
	KH_2PO_4	$MgSO_4$	Peptone	Sunflower oil	(U/mL)
1	-1	-1	-1	-1	4.26
2	1	-1	-1	-1	3.46
3	-1	1	-1	-1	2.14
4	1	1	-1	-1	3.37
5	-1	-1	1	-1	4.98
6	1	-1	1	-1	5.81
7	-1	1	1	-1	3.14
8	1	1	1	-1	4.52
9	-1	-1	-1	1	2.47
10	1	-1	-1	1	2.56
11	-1	1	-1	1	1.59
14	1	-1	1	1	6.43
15	-1	1	1	1	3.85
16	1	1	1	1	4.51
17	-2	0	0	0	2.29
18	2	0	0	0	4.46
19	0	-2	0	0	4.83
20	0	2	0	0	4.25
21	0	0	-2	0	0.56
22	0	0	2	0	2.42
23	0	0	0	-2	6.35
24	0	0	0	2	6.12
25	0	0	0	0	5.97
26	0	0	0	0	5.88
27	0	0	0	0	6.25
28	0	0	0	0	6.09

An analysis of variance (ANOVA) was performed to establish a quadratic surface response model. Table 3 shows the results for the significance level obtained by ANOVA.

Table 3: ANOVA - Analysis of variance for CCRD

Source	Sum of squares (SS)	DF	Mean square (MS)	F test	
				F_{calc}	F_{list}
Model	67.36	16	4.21	12.75	2.31
Residue	5.55	17	0.33		
Total (T)	72.91	27			
R^2 = 0,9298					

Equation 1 shows the lipase activity (Y) expressed as a function of the following components concentrations: KH_2PO_4 ($X1$), $MgSO_4.7H_2O$ ($X2$) and peptone ($X3$) (Eq. 2).

$$Y = 6.27 + 0.50X_1 - 0.71(X_1)^2 - 0.36\ X_2 - 0.42(X_2)^2 + 0.74\ X_3 - 1.18(X_3)^2 - 0.39\ X_2\ X_3 \quad (2)$$

The results shown on Table 3 suggested that the generated model was statistically significant and the lipase activity could be well described with this model ($F_{list} > F_{calc}$). Horizontal bars represent the magnitude of the estimated effects divided by the standard error (absolute value of the effects) while bars crossing dotted line, where $p \leq 0.05$, means statistically significant effects in the confidence limit (95%). These results indicated that peptone; KH_2PO_4 and $MgSO_4.7H_2O$ had significant influence on the lipase produced by *F. verticillioides*. The model adjustment was expressed by the coefficient of determination (R^2 = 0.9298) and revealed a relatively high correlation between experimental and predicted values.

The surface response graphic, obtained from the quadratic model, was generated by the adjusted model (Figure 3) and showed the influence of peptone, KH_2PO_4 and $MgSO_4$ in the enzyme production. It was possible to verify that higher peptone concentrations together with lower $MgSO_4$ concentrations increased the lipase production (Figure 3A). It could also be observed in assay 14 of Table 2, in which was obtained the higher response. It was interesting to observe on Figure 3C, that low that the concentrations of $MgSO_4$ increased the lipase activity. The optimized final medium subtly enhanced the lipase activity by (0.15 % KH_2PO_4, 0.025 % $MgSO_4$, 0.3 % peptone and 1 % sunflower oil). An increase of 4-fold was observed in this study compared to the worst production in the same experiment. The experimental value obtained was 6.430 U/mL (run 14), which was very close to the predicted value of 6.391 U/mL. Good agreement between experimental and predict values were also found by other authors (Lo et al., 2012; Ramani et al., 2013).

Many studies using statistical optimization and RSM model have been employed to predict the combined effects of cultivation conditions. The production depends on the optimum culture conditions of the respective microorganisms. Most of the studies analyzed reported an increase in the enzyme production. Kumari et al. (2009) achieved an increase of 1.4-fold in the lipase activity after medium optimization. Liu and Zhang (2011) and Andrade et al. (2013) increased almost 4-fold in their optimization comparing to the worst production, and a 2.8-fold was obtained by Kaushik et al. 2010.

Crude extract characterization

Effect of pH and temperature on lipase activity and stability

Temperature and pH are effective parameters that could positively or negatively interfere on lipase activity. The results showed a range of optimum pH values varying from 5.5 to 7.0 (Figure 4A) and a temperature of 45°C for lipase activity (Figure 4B). The lipase activity drastically decreased at 70 °C. Acidic pH, could be also observed for the lipase from *Aspergillus oryzae* RIB 128 (Toida et al., 1998). However, many alkali lipases are found in literature (pH > 7.5), working at mild temperatures from different fungal gender (Abbas et al., 2002; Chaiyaso et al., 2012; Hiol et al., 2000; Toscano et al., 2013).

The pH stability (Figure 4 C and A) showed that the lipase was more than 50% stable at a range of pH values between 4 and 8, after 24 h of incubation. Similar stabilities could be observed by *Mucor* sp., *Aspergillus oryzae* and *Rhyzopus oryzae* lipases (Abbas et al., 2002; Hiol et al., 2000; Toida et al., 1998).

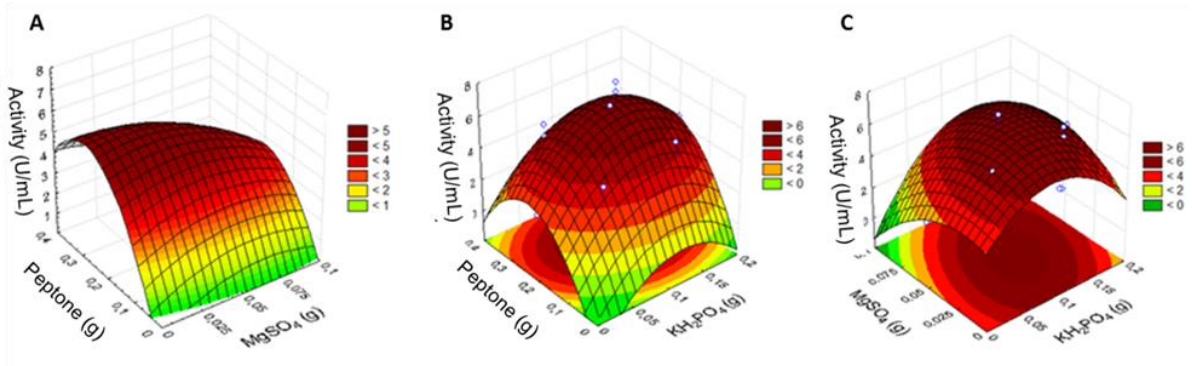

Figure 3 Surface response graphs. (A) Peptone and $MgSO_4$; (B) peptone and KH_2PO_4; (C) KH_2PO_4 and $MgSO_4$ effects on lipase production by *F. verticillioides*.

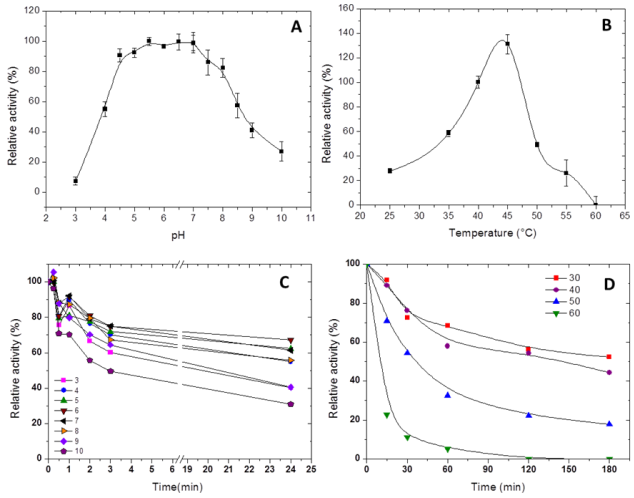

Figure 4: Effect of (A) pH and (B) temperature on lipase activity from *F. verticillioides* and its (C) pH and (D) thermal stability.

Oppositely, results were found by Toscano et al. (2013) reported that lipase from *T. harzianium* was unstable when incubated at pH below 7.0. The newly isolated *F. verticillioides* produced a stable lipase against mild temperatures, retaining more than 50% after 120 min, but not at 50 and 60 °C (Figure 4D). The same result was published by Abbas et al. (2002), in which after 1h incubation the lipase denatured and lost its activity.

One of the reactions that lipases are able to catalyze is that involving organic solvents. It is well known that these compounds can reduce the water activity around the enzyme and promote loss of lipase activity. In this context, the solvent stability of the lipase was studied (data not shown). The results showed 100% stability to DMSO and hexane for 24h, and around 60% for methanol, ethanol, butanol and isopropanol. Chaiyaso et al. (2012) and Abbas et al. (2002) also obtained higher stability in hexane after 24h.

Enzyme application

Lipase hydrolysis of vegetable oils and soapstock oil

Fatty acids are currently produced by chemical processes which are expensive and require elevated temperature and pressure conditions to remove the coloring materials formed. However, these compounds can be enzymatically produced, increasing their purity under mild conditions of temperature and pressure (Abdelmoez et al., 2013).

F. vertillioides lipase is an expressive enzyme used to hydrolyze vegetable oil (Figure 5A). It was able to hydrolyze more than 20% of triacylglycerol present in soybean, sunflower and olive oil, using low crude extracts concentration (17.1%). Abdelmoez et al. (2013) used higher concentration of enzymatic extract (more than 60%) and obtained 39.4% conversion on sunflower hydrolysis. The best conversion time obtained from *F. verticillioides* lipase was 18h, after that a constant of hydrolysis could be observed (Figure 5B).

In our work the soapstock presented a reasonable conversion compared to its control sample (13.3%), but this result may be due to the fact that this residue is essentially consisted by water, sodium salts of fatty acids, triglycerides, phospholipids and products of oil degradation and any component of it could little inhibit the lipase activity.

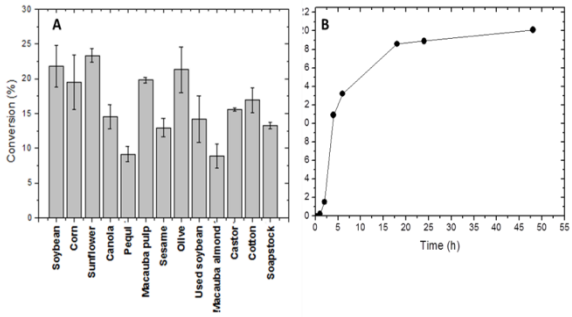

Figure 5: (A) Oil hydrolysis by lipase and (B) time course of sunflower oil hydrolysis.

Fat particles degradation on slaughterhouse wastewater

In general, effluents produced from slaughterhouses, dairy products industry and oil refinery, contain high lipid concentrations. The experiments were carried out with sterilized wastewater in order to investigate only the behavior of *F. verticillioides* lipase in the fat hydrolysis, avoiding interferences caused by the wastewater microflora. Beef tallow and fat were used in this study. Tallows are rendered form of beef fat, processed from suet. The lipase was able to hydrolyze both fat and tallow; however tallow was preferred by the enzyme (Figure 6). Possibly, because of the soft characteristic of this compound in higher temperatures instead of the solid state, as it is at room temperature. It could increase the contact area and make them more available than fats to hydrolyze. The results showed that the free fatty presented in

samples with enzyme were 3-fold higher than the control samples, exhibiting a greater hydrolysis capacity in this kind of waste. Lipases from different sources also presented good results. The lipase from *Pachira aquatica* hydrolyzed fat particles and decreased the mass weight in about 14.3%, after 72h of incubation (Polizeli et al. 2013).

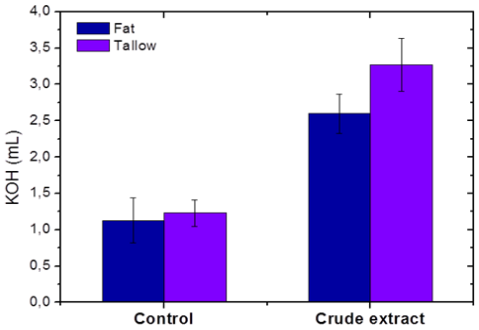

Figure 6: Effect of lipase on fat particles added in slaughterhouse effluent. Fatty acid released was measured by the volume of KOH added. All experiments were performed in triplicate.

Conclusions

The influence of liquid medium, time culture and temperature in SmF was firstly evaluated, and on Adams medium the higher production was reached at 30°C, after 72h. Vegetable oils have been extensively used in lipase production and the presence of sunflower oil, increased the lipase production in approximately 18.5-fold higher than that observed in the worst production (baru powder). Amongst the various nitrogen sources tested, peptone was considered the best. Secondly, the Adams components were analyzed statically in a CCRD. This tool reduced the cost and the number of the experiments required. Thus, efficient lipase production was obtained in a new culture medium composed by: 0.15 % KH_2PO_4, 0.025 % $MgSO_4$, 0.3 % peptone and 1 % sunflower oil. The final lipase activity (6.43 U/mL) was about 2-fold higher on this new medium than the non-optimized medium (3.24 U/mL, after the best nitrogen source selection). The optimization can add value and ensure overall reduction in the enzyme's final production cost. Thus, the R^2 value of 0.9298 showed a good fitting model with the experimental data. These results indicated that the model generated is statistically significant and the lipase activity could be well described by this model.

Apart from these, *F. verticillioides* lipase characterization indicated an optimal temperature and pH of 45°C and 5.5, respectively, and a great stability on a pH range varying from 4.0 to 8.0, for 24 h. These properties are interesting aiming industrial application both in low or high pH range. Moreover, the *F. verticillioides* lipase demonstrated to be a potential enzyme to be applied in oil hydrolysis, releasing a great yield of fatty acids after 18 h. These are important products on the manufacture of coatings, adhesives, biofuels, surfactants, specially lubricating oils, shampoos and other personal care products. Additionally, this lipase was capable to hydrolyze soapstock (13.3%) and reduce fat particles in slaughterhouse wastewater efficiently, in approximately 3-fold. Concluding, this is a potential lipase not only for environmental applications, but also for any other biotechnological procedures as food and cosmetic industries.

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